Understanding the path to a congenital athymia diagnosis

A look at the diagnostic pathway, from newborn screening to confirmation

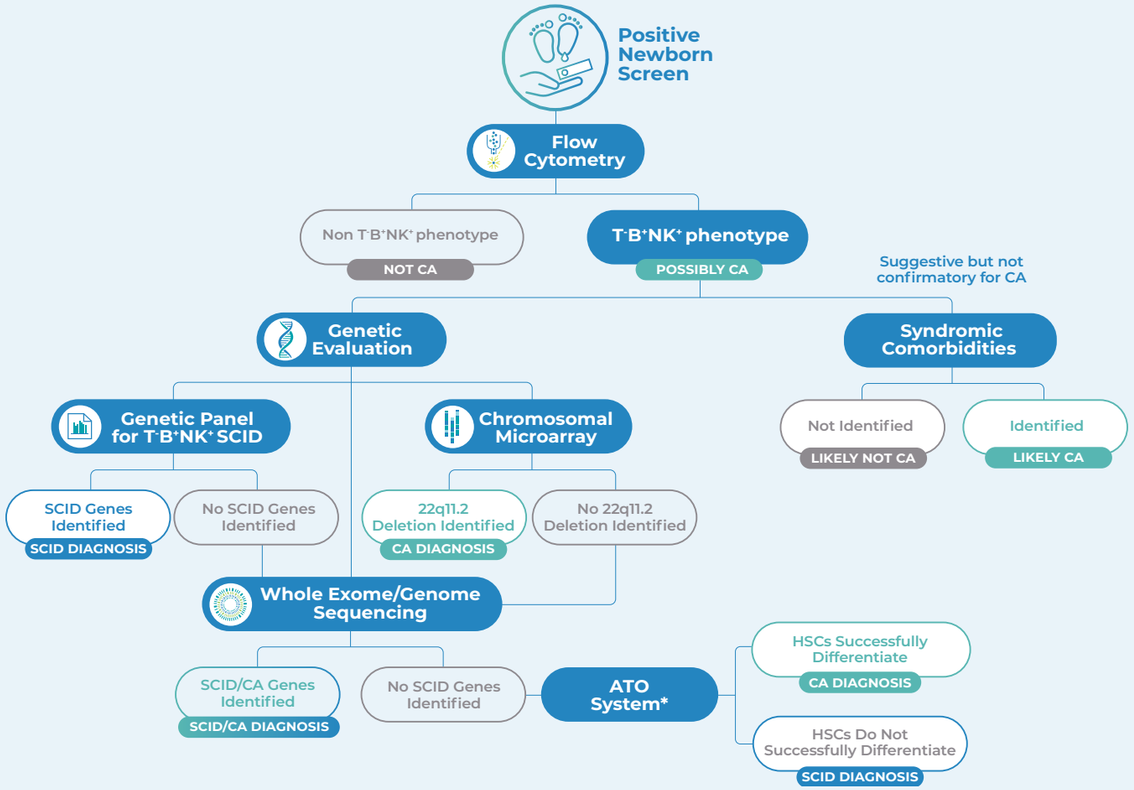
Not an actual patient.

Sumitomo Pharma

Diagnosing congenital athymia can be a complex process[1]

Newborn screening for severe combined immunodeficiency (SCID) plays a crucial role in the early detection of congenital athymia, a primary immunodeficiency (PI) characterized by the lack of a functioning thymus at birth. Distinguishing between SCID and congenital athymia is critical as they have different underlying causes and treatment requirements. **Early diagnosis is key to making the right supportive care decisions.**[1,2]

There are a number of laboratory tests that can be performed to differentiate between congenital athymia and SCID to get you closer to confirming the proper diagnosis. However, the pathway to diagnosis varies from patient to patient and may not follow all of these steps.[1]



Collins C, Sharpe E, Silber A, Kulke S, Hsieh EWY. Congenital athymia: genetic etiologies, clinical manifestations, diagnosis, and treatment. *J Clin Immunol.* 2021;41(5):881-895. doi:10.1007/s10875-021-01059-7

LEGEND

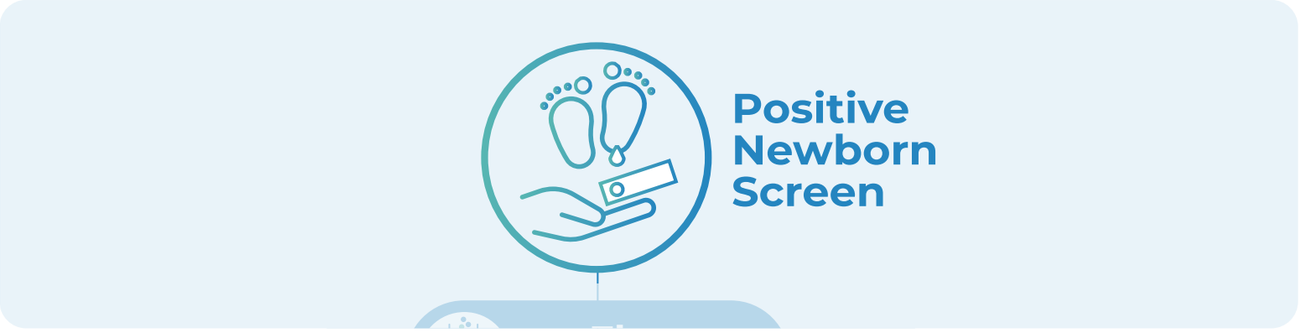
CA	Congenital Athymia
SCID	Severe Combined Immunodeficiency
ATO	Artificial Thymic Organoid
HSC	Hematopoietic Stem Cell

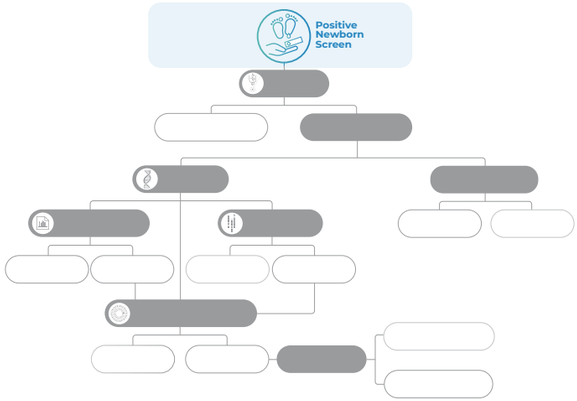
*Investigational test performed at the National Institutes of Health (NIH).

Key consideration

Determining a diagnosis of congenital athymia or SCID is a multistep process that should be followed with the guidance of a pediatric immunologist.[1]

A positive newborn SCID screening result is typically the first indication of congenital athymia[1]





Newborn screening for SCID—a test mandated in all 50 states—often provides **the first signal of a congenital athymia diagnosis**. This test measures immune function by evaluating T cell receptor excision circles (TRECs).[1]

TRECs are formed during T cell receptor rearrangement in the thymus. **Low or undetectable TRECs are considered a positive finding**, making it an early indicator for congenital athymia.[1]

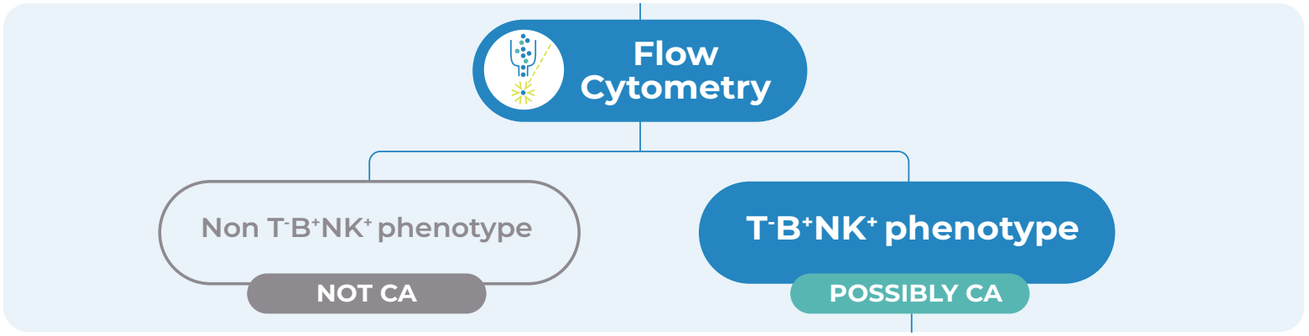
Upon receiving a positive newborn SCID screening result, **consult a pediatric immunologist about the measures that should be taken to protect your patient.** These measures may include[3]

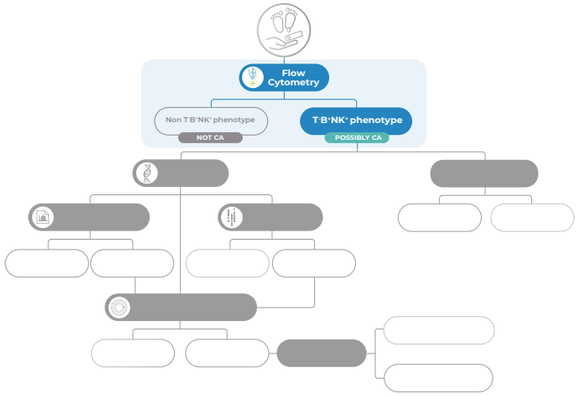
- Beginning protective isolation
- Stopping breastfeeding
- Suspending vaccinations

Key considerations

Patients who receive a positive newborn SCID screening result require immunophenotyping via flow cytometry to test their blood for the presence of naive T cells, as well as B cells and NK cells.[1]

Phenotyping via flow cytometry





After a patient has received a positive newborn SCID screening result, **complete and differential blood count tests and lymphocyte phenotyping by flow cytometry should be performed**. The lymphocyte phenotype is determined by the presence of T cells, B cells, and Natural Killer (NK) cells.[1]

Patients with congenital athymia may or may not have low total T-cell counts, but they do have profoundly low naive T-cell counts—either **fewer than 50 naive T cells/mm³ or less than 5% of their total T cells are naive**. They have normal amounts of B cells and NK cells, though.[1]

These patients will present with a $T^-B^+NK^+$ phenotype, but more testing will be required to differentiate their condition from $T^-B^+NK^+$ SCID.[1]

Identifying typical vs atypical congenital athymia

Patients diagnosed with typical congenital athymia may, over time, develop atypical congenital athymia. These two phenotypes have different characteristics, which include[4]

Typical congenital athymia

- T-cell lymphopenia
- Absence of rash or lymphadenopathy
- Lack of mitogen-stimulated T-cell proliferation

Atypical congenital athymia

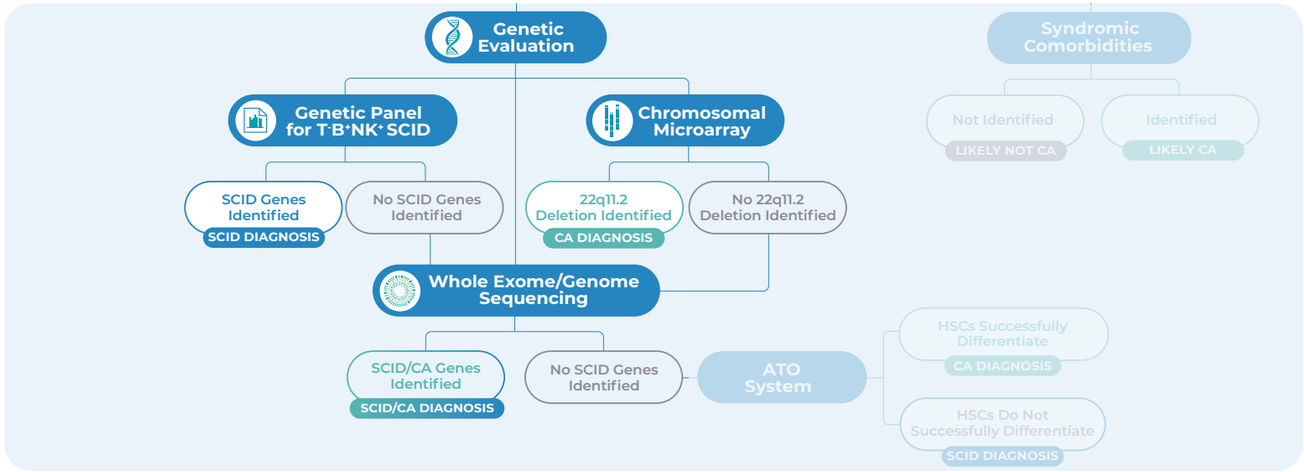
Signs and symptoms of autologous graft versus host disease (GVHD):

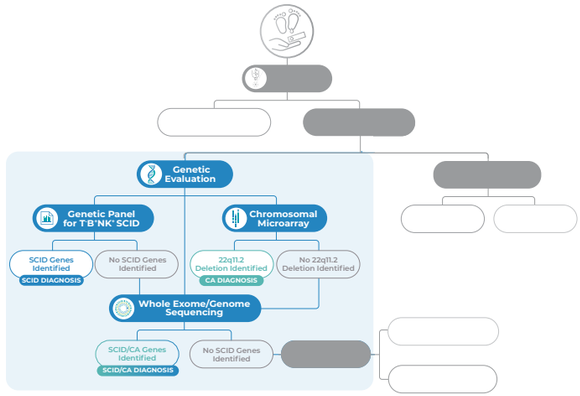
- Rash
- Lymphadenopathy
- High numbers of circulating T cells (from oligoclonal T-cell expansion)
- T-cell proliferation in response to mitogens (eg, phytohemagglutinin)

Key consideration

Patients with congenital athymia **may or may not** have low total T-cell counts, but **will have** profoundly low naive T-cell counts.[1]

Looking deeper through genetics





Differentiating between congenital athymia and T⁻B⁺NK⁺ SCID will require looking for **known mutations in the genes that cause these conditions.**[1]

Genetic testing to confirm or rule out T⁻B⁺NK⁺ SCID must be performed. These tests may include a genetic panel for T⁻B⁺NK⁺ SCID, a chromosomal microarray analysis, and whole exome or genome sequencing, which may be used to determine the presence of these known genetic mutations.[1]

Genetic panel for T⁻B⁺NK⁺ SCID[1]:

- The presence of known mutations in genes such as *IL7R* or *CD3D* can confirm a SCID diagnosis

Whole exome/genome sequence[1]:

- Can identify known mutations to genes that cause either SCID or congenital athymia, leading to a diagnosis of either one

Chromosomal microarray analysis[1]:

- Can determine if part of chromosome 22 is missing—which includes the *TBX1* gene
- This will confirm a diagnosis of 22q11.2 deletion syndrome (DiGeorge syndrome), which indicates an increased likelihood of congenital athymia when combined with a positive newborn SCID screening result

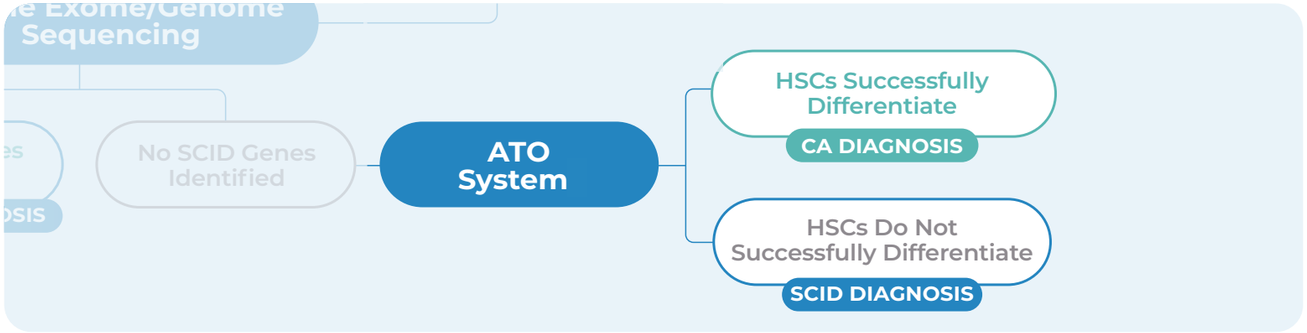
If no known mutations are found, further testing must be performed to confirm a diagnosis.[1]

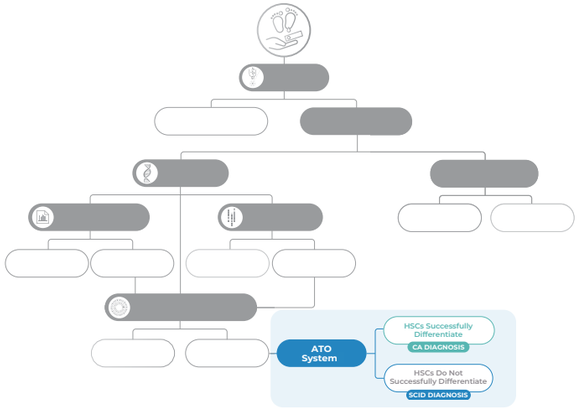
Key considerations

Some genes that have been implicated in the development of congenital athymia and T⁻B⁺NK⁺ SCID include[1]

- *FOXN1, PAX1, TBX1, CHD7, FOXI3, TBX2* (congenital athymia)
- *IL7R, CD3D* (T⁻B⁺NK⁺ SCID)

Differentiating with an artificial thymic organoid (ATO)





If a diagnosis is still unclear after genetic evaluations, an artificial thymic organoid (ATO) can be used to **test the ability for hematopoietic stem cells (HSCs) to become mature T cells.**[1]

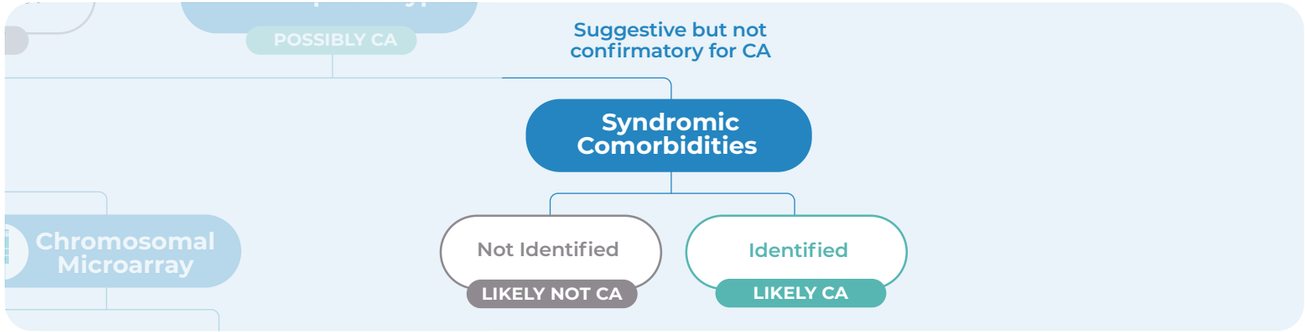
ATO systems can support in vitro differentiation, positive selection, and maturation of human T cells from cord blood, bone marrow, and peripheral blood $CD34^+$ HSCs. ATO system analysis may help determine if the issue is with the thymus or with the HSCs in the bone marrow.[1,5]

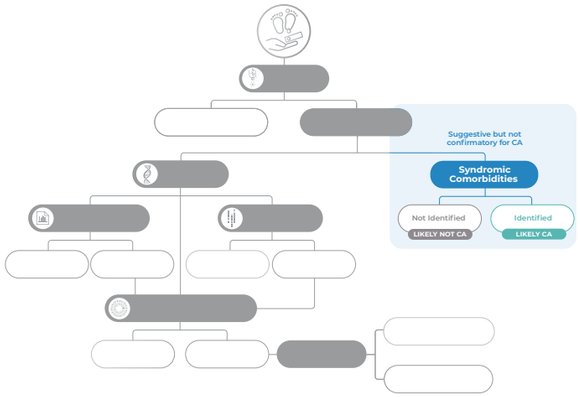
A congenital athymia diagnosis is supported by the successful differentiation of HSCs from the patient using the ATO assay. If they do not differentiate successfully, a diagnosis of SCID may be confirmed.[1]

Key consideration

Testing for congenital athymia versus SCID using an ATO is performed as a research test at the National Institutes of Health (NIH).

Assessing associated conditions





Congenital athymia has previously been referred to as complete DiGeorge anomaly, but has since been associated with other syndromic comorbidities, including **genetic conditions, congenital syndromes, and environmental exposures**. Some of these conditions include[1,4]

Complete DiGeorge syndrome (22q11.2 deletion syndrome)[1]

- Associated with missing genetic material from chromosome 22
- May result in a compromised immune system, heart defects, developmental delay, hearing loss, and other conditions

CHARGE syndrome[1]

- Associated with mutations in the *CHD7* gene
- Differs depending on the patient, but the most common features are coloboma, heart defects, atresia of the nasal choanae, retardation of growth and development, genitourinary anomalies, and ear anomalies

FOXN1 deficiency[1]

- Affects the production of the FOXN1 protein, which is critical to embryonic development of thymic epithelial cells (TECs)
- May result in a severely compromised immune system and problems with the growth of hair and nails

Diabetic embryopathy[1]

- Abnormal thymic development has been seen in infants of diabetic mothers
- May be associated with altered fetal thymus size and other congenital abnormalities

Key consideration

Identifying a syndromic comorbidity may suggest a diagnosis of congenital athymia but is not adequate to confirm a diagnosis on its own.[1]

This is not an exhaustive list of conditions associated with congenital athymia. Evaluations must always be made on an individual basis as clinical manifestations may vary from patient to patient.

Patients with congenital athymia require vigilant care

Once a diagnosis is confirmed, your patient should be enrolled in the support program, referred for treatment, and started on supportive care.

Supportive care includes continuing the infection prevention measures that began when the positive newborn SCID screening result was confirmed, as well as the following[3]:

Immunoglobulin (IgG) replacement therapy[1]

Prophylaxis for *Pneumocystis jirovecii*[6]

Antibiotic, antimicrobial, and antifungal prophylaxis[1]

Monitoring for signs of infection and autologous GVHD[1,3]

This is not an exhaustive list of supportive care measures. Additional supportive care measures may vary from patient to patient based on comorbidities.

To enroll your patient in the support program, visit
congenital-athymia.com/enroll

Diagnostic Summary

You can use this summary of tests to help track your patient's progress along the diagnosis journey. However, keep in mind that the pathway to diagnosis varies from patient to patient and may not follow all of these steps. **A patient who receives a positive newborn SCID screening result should be referred to a pediatric immunologist to determine the appropriate care plan.**[1,3]

		Not congenital athymia	Possibly congenital athymia*	Confirmed congenital athymia
Flow cytometry	$T^-B^+NK^+$ phenotype†		X	
	Not $T^-B^+NK^+$ phenotype	X		
Genetic panel for $T^-B^+NK^+$ SCID This test must always be performed to rule out the possibility of other conditions.	SCID genes identified	X		
	No SCID genes identified		X	
Chromosomal microarray	22q11.2 deletion identified‡		X	
	No 22q11.2 deletion identified		X	
Whole exome/genome sequencing	SCID genes identified	X		
	Congenital athymia genes identified			X
	No clear genetic cause identified		X	
ATO system analysis§	HSCs successfully differentiate			X
	HSCs do not successfully differentiate	X		
Syndromic comorbidities	Identified		X	
	Not identified		X	

*These results should not be used to confirm a diagnosis of congenital athymia. Further testing will be required.
†Patients with congenital athymia may or may not have low total T-cell counts, but will have profoundly low naive T-cell counts.[1]
‡A diagnosis of congenital athymia may only be confirmed if the genetic panel for $T^-B^+NK^+$ SCID did not identify any SCID genes.
§An ATO system analysis may not always be able to provide a definitive diagnosis.

References

1. Collins C, Sharpe E, Silber A, Kulke S, Hsieh EWY. Congenital athymia: genetic etiologies, clinical manifestations, diagnosis, and treatment. *J Clin Immunol*. 2021;41(5):881-895. doi:10.1007/s10875-021-01059-7 **2.** Immune Deficiency Foundation. *Patient & Family Handbook for Primary Immunodeficiency Diseases*. 6th ed. 2019. **3.** Gupton SE, McCarthy EA, Markert ML. Care of children with DiGeorge before and after cultured thymus tissue implantation. *J Clin Immunol*. 2021;41(5):896-905. doi:10.1007/s10875-021-01044-0 **4.** Markert ML, Gupton SE, McCarthy EA. Experience with cultured thymus tissue in 105 children. *J Allergy Clin Immunol*. 2022;149(2):747-757. doi:10.1016/j.jaci.2021.06.028 **5.** Bifsha P, Leiding JW, Pai SY, et al. Diagnostic assay to assist clinical decisions for unclassified severe combined immune deficiency. *Blood Adv*. 2020;4(12):2606-2610. doi:10.1182/bloodadvances.2020001736 **6.** Markert ML. Defects in thymic development. In: Sullivan KE, Stiehm ER, eds. *Stiehm's Immune Deficiencies: Inborn Errors of Immunity*. 2nd ed. Elsevier; 2020:357-379.

Sumitomo Pharma